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Immunological approaches to fertility regulation

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In view of strong evidence that there are specific components of the reproductive system that are not represented in other body systems, and that many of these are immunogenic, efforts are being made to develop an acceptable vaccine for fertility regulation. It is hoped that such a vaccine could be administered at infrequent intervals by specially trained technicians in regions without clinics or medical facilities. For safety and practical reasons, an approach using active immunization procedures with a vaccine is preferred to passive immunization with antibodies.

In experiments with sperm antigens, a lactate dehydrogenase isoenzyme (LDH-X), one of the enzymes normally present on the sperm surface, has reduced fertility in mice and rabbits; there was, however, significant embryo mortality and further study is required. Other sperm antigens have also been tested but most have been rejected. Most of the research on ovum antigens has been directed towards the zona pellucida, and work is in progress at many centres to isolate experimental quantities of specific zona pellucida antigens. Antibodies to human zona react with pig zona and vice versa *thus providing a model system to evaluate such antigens.*

Antibodies to whole-placenta homogenates disrupt pregnancy in several species of laboratory animal and two of the placenta-specific proteins have been evaluated as potential antigens, since antibodies to them do not react with any other tissue so far tested. Three protein hormones have been isolated from placental tissue and two have been studied as potential antigens. The problems of testing the safety of possible antigens require extensive study.

Studies over the past two decades have provided strong evidence that there are specific components of the reproductive system that are not represented in other body systems and that many of these are immunogenic. Other studies in animals have shown that antibodies to reproductive antigens are capable of preventing or disrupting gestation. While the action of such antibodies has generally not been well defined, it is believed that the functions of certain physiologically active substances, particularly proteins, can be blocked immunologically. If it were possible to prepare well defined reproductive antigens, essential to successful reproduction, capable of eliciting an appropriate immune response when injected into human subjects and not provoking responses affecting nonreproductive systems, an acceptable vaccine for fertility regulation would be available.

Immunological methods of fertility regulation are one of the priority areas of the WHO Special Programme of Research, Development and Research Training in Human Reproduction and many of the studies described in this article were planned and supported by the Special Programme. This multidisciplinary research effort, in which scientists and institu-

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tions from about 70 countries are collaborating, places great emphasis on research in fertility regulation, but also includes research in such areas as infertility, maternal mortality, and pregnancy wastage.

RATIONALE OF THE IMMUNOLOGICAL APPROACH

The principal advantage of an immunological approach to fertility regulation is the potential for infrequent administration of an effective method by specially trained technicians who could deliver antifertility services to a large number of people in regions without clinics or other medical facilities. Also, antigenic materials eliciting immune responses blocking physiologically active reproductive components need not be active themselves since responses can often be obtained with molecules made nonactive by chemical alteration or by using only fragments of the active component. In this way, side-effects often encountered with other methods using pharmacologically active compounds can be avoided.

To obtain an antigen for vaccine development, many factors must be considered. Firstly, the substance to be inhibited by the immune response should ideally be present in the vaccine recipient only intermittently (i.e., during pregnancy or intercourse) and the mass of antigen to be blocked should be relatively small. Quantities of antigen in a highly purified state must be available at a reasonable cost if application of the vaccine to a large population is to be accomplished. This latter requirement limits, for many reproductive substances, the possibility of isolating vaccine antigens from natural sources unless immunological similarities are found between human and animal components. Certain antigens from human placentae are exceptions, but sufficient supplies could not be obtained of human sperm or eggs for large-scale vaccine production. As a general rule, antigenic components would need to be chemically defined so that synthetic production would be feasible. In some cases, only portions of the natural molecule to be inhibited can be used as an immunogen for inducing a specific immune response. For fertility regulation in the female, these criteria are met by some antigens of placental hormones, by certain non-hormonal plancental glycoproteins, and by sperm antigens.

The emphasis of the research effort to date has been on developing a method for immunizing women to prevent or disrupt fertility. This is because the blocking of components of placentae or sperm by a maternal immune response would appear to be a safe way of interfering with reproductive function. Certain antigens from both sources are not normally present in women and antibodies generated to them would react with " foreign " components when they are encountered. In contrast, no such foreign components are essential for normal reproductive function in men. Attempts to inhibit fertility in human males by immune responses are much more likely to produce damage to reproductive organs or to produce undesirable side-effects. Should data become available from animal studies suggesting that safe procedures for males might be feasible, such approaches could then be actively pursued.

The immunological mechanism generally chosen as a potential antifertility method has been the development of active immunization procedures to produce a vaccine. This approach, as opposed to passive immunization with antibodies, is based on safety considerations and the practical use of the method. Although it is possible to produce highly specific antibodies in animals capable of interfering with human reproduction, their

repeated administration is considered hazardous by most experts. Sensitization of humans to animal immunoglobulins after the initial treatment makes severe reactions to subsequent treatments possible, if not probable. Also, the life of effective antibody concentrations in treated persons would not be more than a few weeks or months. Thus, antibody administration would have to be repeated at relatively short intervals and the main advantage expected of active immunization would not be obtained. Passive immunization would have the advantage that antibodies used could be well characterized before use and that the effects of treatment could be reversed simply by discontinuing therapy. Should new data suggest safe means of passively immunizing humans with animal sera, this approach could be re-evaluated.

A major difference exists between a vaccine to control an infectious disease and one to regulate human fertility. Current vaccines for preventing illnesses by bacteria, viruses, or foreign toxins have the advantage that the targets of the immune response are nonhuman pathogens to which immunity is readily generated in immunocompetent individuals, while an antifertility vaccine must be capable of inhibiting the function of a human substance, although it may be a " non-self " component. Although " non-self " antigens such as sperm or placental proteins introduced into women are foreign to the maternal environment, it is not straightforward that an immune response to them will be elicited. On the contrary, the immunological privilege of these components that result in fertilization and a surviving conceptus during normal pregnancy remains one of the least well explained phenomena in biological science. Thus, the mere identification and isolation of a specific reproductive antigen meeting the criteria discussed above, does not necessarily provide a suitable immunogen for a vaccine, procedures must be developed to render antigenic components that are normally nonantigenic. Whether this will consist of breaking naturally acquired tolerance or inducing " cross-reactive " immunity to natural antigens will depend upon the component involved and the normal mechanisms of its immunological privilege.

Another difference between antifertility immunization methods and conventional vaccination is that with the latter little concern is usually given to the termination of immunity. Ideally, antifertility vaccination should be reversible to permit child-bearing at predictable intervals. This requirement would preclude enhancement of immunity by exposure of the immune system to natural antigens (i.e., to sperm antigens during intercourse or placental antigens during pregnancy). One must induce an immune response capable of blocking a natural human component that cannot be " boosted " by the same natural component and, clearly, chemically altered antigens must be used. However, even if permanent sterility were the only option from such a method, immunization would be more acceptable than the surgical sterilization procedures now in use. Despite the many obstacles to the development of such a method, intensive research is in progress to devise methods for human application; some of the findings of this research are summarized below.

STATUS OF CURRENT RESEARCH

Sperm antigens

The idea that antifertility could be induced in women by the production of antibodies to sperm or other semen components has prevailed for at least 50 years. Antisperm could block fertility by causing immobilization or death of spermatozoa, interference with sperm transport, increased phagocytosis in the female genital tract, or interference with sperm–

ovum contact. During the second and third decades of this century, claims were made of induced infertility following injections of semen into women. However, since there was no evidence that specific immune responses were the cause of infertility in these subjects, enthusiasm for this means of fertility regulation decreased.

These early efforts did, nevertheless, provide a background and basis for the research currently in progress. However, since many problems are associated with active immunization with whole semen or spermatozoa, sperm-specific soluble antigens are considered to be the most suitable immunogens for possible use in an antifertility vaccine. Notable among these are enzymes normally present on the sperm surface. A lactate dehydrogenase isoenzyme (LDH-X) has been purified from mouse testes and antibodies to it have been shown to cross-react with analogous enzymes in several species, including man. No immunological cross-reaction has been found with other body tissues in the mouse or rabbit. Active immunization of female mice and rabbits with LDH-X has reduced fertility (Table 1). However, a significant rate of embryo mortality was observed in immunized animals. A correlation of antibody levels and fertility reduction was observed, and should a means be found to enhance antibody production, complete inhibition of fertility might be achieved. The use of this antigen is attractive since much of its primary structure has been identified and synthesis of antigenic peptides may soon be accomplished. The observation of embryo mortality in immunized animals may represent a serious drawback to use of this antigen in humans. Studies are in progress utilizing subhuman primates as test animals and employing various techniques to improve antibody responses.

Acrosomal hyaluronidase has a vital physiological role in the dispersal of the cumulus oophorus surrounding the ovum before fertilization. This enzyme has been purified from ovine, bovine, and rabbit spermatozoa and is also being studied as a potential antisperm immunogen. Antibodies to rabbit sperm hyaluronidase show no cross-reactivity with other body substances, and there is a high degree of species-specificity. These antibodies agglutinate and immobilize rabbit sperm in the presence of complement and *in vitro* fertilization of rabbit ova is markedly reduced by antihyaluronidase isoantibodies. However, isoimmunization of female rabbits and sheep has failed to produce a significant *in vivo* antifertility effect. Such negative findings may have been related to immunization techniques. However, the species-specificity of this sperm enzyme makes studies in subhuman primates very difficult.

Still another sperm enzyme, acrosin, an acrosomal proteinase, was once thought to offer promise as an antigen for an antisperm vaccine. Purification of this substance from boar,

Table 1. Fertility of female rabbits immunized with mouse LDH-X [a]

Treatment group	No. of animals	Corpora lutea	Blastocysts (day 6 p.c.)	Implants (day 10 p.c.)	Ratio [b] (× 100)
Control	15	127	94	—	74.0
	13	126	—	95	75.4
Immunized	10	108	44	—	40.7
	23	273	—	120	43.9

[a] For details of methodology see Goldberg, E. *Science,* **181** : 458-459 (1973).
[b] Blastocysts/C.I. or Implants/C.I.

ram, and human gametes was accomplished and antisera to the respective preparations were tested. These antisera suggest species-specificity and tests with human and sheep anti-acrosin sera initially showed no immunological reactions to a variety of body tissues and fluids. The enzymatic activity of acrosin is readily inhibited by antisera and in preliminary experiments immunization of female sheep with purified ram acrosin caused a reduction in fertility. Subsequent testing failed to confirm these antifertility effects and additional discouragement arose when evidence was found that this enzyme was in fact not sperm-specific.

These sperm antigens are not the only ones that have been studied as immunogens. Numerous others have been investigated, including two antigens called S and T isolated from guinea-pig spermatozoan membrane; sperm-immobilizing antigen from several species; and human sperm membrane carbohydrates. Despite the immense effort expended in attempting to identify a suitable sperm antigen for use as an immunogen to regulate fertility, most of the candidates have now been rejected. There is still hope that LDH-X or perhaps a sperm immobilizing antigen may be suitable. Data are now available indicating that as many as thirty separate glycoproteins are surface membrane components on normal sperm. Some of these have been isolated and partially characterized. Further study of such cell membrane antigens could reveal new leads to the long-sought sperm antigen for use in a vaccine. It is now apparent that a careful systematic approach to the isolation of sperm antigens is required before the identification of an immunogen for a vaccine can be anticipated.

Ovum antigens

Theoretically, the ovum is an ideal target for interference with the reproductive process. In humans, usually only one ovum is present in the female genital tract at a time and it remains there for several days before implantation. Also, there are numerous cells, membranes, and coatings associated with the ovum containing components that could be antigenic. Antibody action resulting in the non-viability of the unfertilized egg, either in the ovary or after ovulation, would prevent fertilization. This early effect on fertility would have an advantage over disruption of gestation at a later stage from both the medical and religious viewpoints.

Despite the attractiveness of ovum antigens for use in a vaccine, one must remember that the ovum is a maternal component and that immunization of women with " self " antigens could result in autoimmune damage to normal body constituents. Even if antigens could be found that are ovum specific, antibodies to them in women could cause damage to ovarian tissue by virtue of the formation of antigen–antibody complexes on unovulated ova. Furthermore, it may not be easy to render " self " antigens antigenic and the use of cross-reacting antigens from animal sources may impart additional hazards. While careful attention must be given to the safety of this approach to vaccine development, the potential advantages of ovum antigens make studies in this area worth while.

Components of the cumulus and corona cells as well as of the egg surface have been found to be antigenic by heterologous immunizations but antibodies to them have shown little effect upon egg viability. In addition, antibodies to these components of ova react with somatic tissues, an observation directly contraindicating their use for human immunization. Work with cumulus and corona cell antigens has, however, been very preliminary and further study may be more encouraging.

Most research on ovum antigens is directed toward the zona pellucida. This acellular, gelatinous layer surrounding the ovum offers perhaps the most promise as a source of antigens for specific immunological inhibition of ovum viability. The functions of the zona pellucida include mechanical protection of the egg, osmotic regulation, prevention of polyspermy, species specificity of fertilization, and support of blastomeres. The zona is essential for sperm recognition and attachment prior to fertilization. While no specific components have been isolated from this structure, antibodies specific to it have been prepared. After absorption of antibodies to whole ovaries with various tissues not containing ova, sera have been obtained that react only with zona. Such antibodies block fertility in several rodent species, sometimes for long periods. The mechanism of fertility inhibition could be either the prevention of sperm penetration or, if fertilization has occurred, the blocking of implantation by interfering with zona shedding.

Work is in progress in several centres to isolate experimental quantities of specific zona pellucida antigens. This effort has been encouraged by the observation that antibodies to porcine zona react with zona from human subjects and non-human primates. Antibodies to human zona likewise react with pig zona. These findings provide a model system in which to evaluate the efficacy and safety of zona immunization for fertility regulation. Following the preparation of sufficient quantities of zona antigens, intensive studies could be performed to investigate such vital questions as antibody specificity, mechanisms of fertility disruption by antibodies, and the consequences of active and passive immunization of animals against specific zona antigens.

Placental antigens

The human placenta synthesizes a large number of substances: many of these are not represented in either the maternal or paternal organism and some are known to have important physiological roles in the maintenance of pregnancy. Some other substances isolated from placental tissue have no known function since our general understanding of placental physiology remains sketchy. However, should one or more of these components be vital to the survival of a conceptus, it would seem reasonable to expect that pregnancy could be disrupted by immunological means. Certain placental antigens are expressed prior to implantation and are contained in the differentiated blastocyst. These materials are believed to be of trophoblast origin and may be important to the protection of the developing conceptus from maternal immunological surveillance. These facts, as well as the knowledge that placental proteins are genetically foreign to the female, has stimulated research to identify potential antigens from this source for a fertility regulating vaccine.

Antibodies to whole placenta homogenates disrupt pregnancy in several species of laboratory animal. In most studies, the antibodies produced were not specific to the placenta and whether disruption of fertility was related to placental antibodies or non-specific factors could not be ascertained. In recent years, progress has been made toward the isolation of highly purified proteins from placental tissue. Several of these are placenta specific and individually or grouped they have been used in efforts to develop an antifertility vaccine.

Most of the placenta-specific proteins identified so far are listed in Table 2. Immunochemical studies of the placental enzymes have revealed that antibodies to these will react with enzymes in other tissues. They are also present in very low quantities in term placentae. Two of the proteins with unknown function, SP-1 and PP-5, have been evaluated

Table 2. Characteristics of the placenta-specific proteins

Antigen	Physico-chemical properties			Amounts in which they occur in:	
	Molecular weight	Chemical nature	Carbohydrate content (%)	Term placenta (mg/placenta)	Pregnancy sera (mg/100 ml)
human chorionic gonadotropin	47 000	glycoprotein	31	< 1	< 1
human chorionic somatomammotropin	21 600	protein	0	150	< 1
human chorionic thyrotropin	25 000	glycoprotein	4.5	< 1	?
heat-stable alkaline phosphatase	70 000 116 000	glycoprotein	?	400	< 0.1
cystine aminopeptidase	290 000	glycoprotein	44	?	1
17 β-hydroxysteroid dehydrogenase	68 000	protein	0	4	?
SP1	90 000	glycoprotein	28	30	5-30
PP5	50 000	glycoprotein	10	2	< 0.1

[a] Data from Bohn, H. (Bibliography, 2).

as potential antigens since antibodies to them do not react with any other tissue so far tested. SP-1 is produced in large quantities by the placenta and is secreted in increasing amounts during gestation. It can be detected in blood at three weeks after conception and reaches levels exceeding 100 μg/ml before term. Homogeneous preparations of SP-1, a glycoprotein with a molecular weight of about 90 000, have been prepared and immunological studies for antifertility effects performed.

Passive immunization with antisera raised in rabbits to human SP-1 induced abortions in 8 of 10 pregnant cynomolgus monkeys between day 19 and day 55 of gestation. Abortions were also produced in baboons using sheep antihuman SP-1. Fewer effects on fertility were observed when monkeys and baboons were actively immunized with human SP-1 prior to mating. While approximately half of some 25 monkeys and 10 baboons aborted after active immunization, the time of abortion varied from 20 to 110 days of gestation. There was no correlation between the occurrence or time of abortion and antibody titre. The only explanation for these variable findings was the observation that antihuman SP-1 showed only partial cross-reaction with a similar substance isolated from monkey and baboon placentae. If the antibodies raised to human SP-1 in some of these primates were not capable of neutralizing endogenous SP-1, no abortion would have occurred despite high antibody levels to the human antigen. In order to answer the question of whether species-specificity or nonspecific effects from passive immunization accounted for these variable results from heterologous immunizations, SP-1 was prepared from monkey placentae in sufficient quantity to isoimmunize female monkeys. Repeated attempts to induce antifertility by immunization of nonhuman primates have failed. While antibodies were generated in response to injections of chemically altered monkey SP-1 into monkey and baboons, pregnancy was not interrupted in either species. Analysis of hormone and antibody levels in immunized baboons revealed that antibody levels dropped sharply following conception and serum levels of SP-1 were reduced below normal for only a short period. These observations suggest that the quantity of SP-1 produced by the

placenta is too great to be blocked by the levels of antibody generated by active immunization. This finding leaves little hope that SP-1 can be employed for the development of a suitable vaccine.

Much less work has been done with the placental protein identified as PP-5 since its recovery from term placentae is very low (about 2 mg/placenta). On the other hand, PP-5 is not secreted during pregnancy and, therefore, lower quantities of antibodies may be effective in blocking the action of PP-5. Of course, it is not known whether PP-5 has a vital role in pregnancy maintenance and antibodies to it may have no effect on the course of gestation. Despite these uncertainties, human PP-5 has been used to study antifertility effects of immunization in female monkeys. Although the results are preliminary, a significant reduction in fertility has been observed in treated animals. Twenty matings of 11 cynomolgus monkeys injected with chemically altered PP-5 resulted in 3 pregnancies in comparison with 5 pregnancies from 9 matings of 5 monkeys in the control group. While this pregnancy rate is unacceptable for human application, further attempts to improve immune responses, particularly to isoimmunization of primates, seem justified before this approach to vaccine development is abandoned.

Three protein hormones have been isolated from human placental tissue: chorionic thyrotropin (hCT), chorionic gonadotropin (hCG), and chorionic somatomammotropin (hCS). Both hCG and hCS have been studied as potential antigens for antifertility immunization. Although much is known about the chemistry and function of these placental substances, their similarity to pituitary hormones leads to concern regarding the specificity of antibodies raised to them. This lack of specificity has been a major problem throughout investigations of these substances. Antibodies to hCS, passively administered to pregnant rats, rabbits, and baboons have consistently produced abortions. Rats and rabbits actively immunized with the human antigen have also exhibited reduced fertility. Based upon these findings, highly purified CS was prepared from baboon placentae and fertile female baboons were immunized with hapten-coupled CS. Although the resulting antibodies showed neither significant reaction with baboon growth hormone nor reduced serum levels of growth hormone or somatomeden, subsequent pregnancies in these females were not disrupted. Antibody levels in pregnant animals disappeared when significant placental secretion of CS began and serum CS levels remained normal throughout gestation (Fig. 1). The effects of CS immunization on pregnancy in baboons are virtually identical to those of SP-1 immunizations described earlier. Although CS is not detected in the blood of pregnant baboons as early as SP-1, it is secreted in large amounts. Also like SP-1, it appears unlikely that hCS can be used as an antigen to develop an antifertility method.

There is more hope with hCG of finding an appropriate antigen for use in a vaccine. Although antibodies to intact hCG raised in laboratory animals and human subjects react significantly with human pituitary luteinizing hormone (LH), antisera to the beta subunit of hCG (β-hCG) react with hLH to a lesser degree. While with most antisera to β-hCG the reactivity to hLH is about 10% of that to hCG, some antisera to β-hCG raised in rabbits have as little as 1% cross-reaction with hLH. However, no antisera have yet been obtained following immunization of rats, rabbits, sheep, or baboons that did not show some reaction with purified hLH. However, women have been immunized with chemically altered β-hCG and no alteration of serum hLH levels or of events of the menstrual cycle were reported. Active immunization of 10 female baboons and passive immunization of pregnant baboons with anti-β-hCG serum has demonstrated complete disruption of early pregnancy by antibodies to the hormone subunit. Thus, the efficacy of this substance as an immunogen is

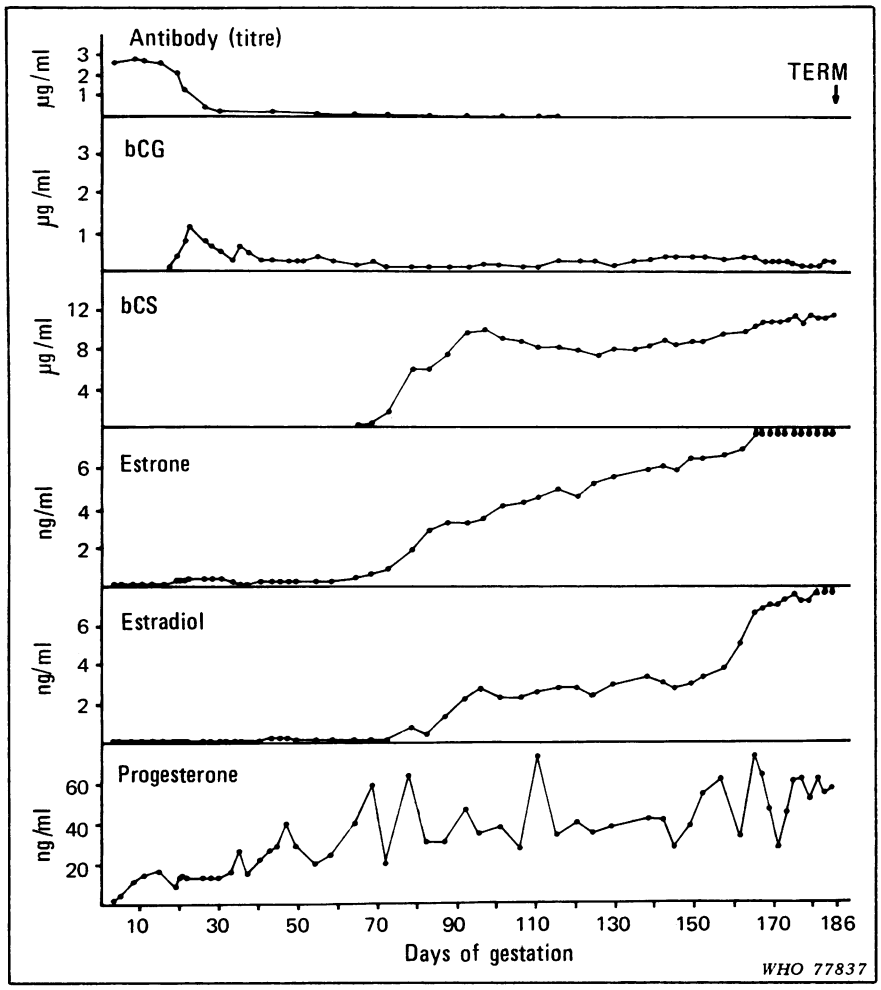

Fig. 1. Hormone and antibody levels during pregnancy in a baboon actively immunized with baboon chorionic somatomammotropin (bCS).

well established; however, there is controversy as to whether its safety has been sufficiently tested to warrant studies in humans.

Examination of the primary structures of the beta subunits of hCG and LH reveals that there may be a way to overcome the problem of obtaining specific hCG antibodies. There is a 33-amino acid sequence at the C-terminal end of β-hCG that is not represented in the hLH molecule (Fig. 2). Peptides from this region of the molecule have been obtained by degradation of natural hCG and by synthetic processes. Since these are small molecules with a molecular weight of about 3000, peptides must be conjugated to larger carrier substances in order to enhance their antigenecity. Such conjugates have been prepared and antibodies have been raised to them in laboratory animals. Antibodies directed to some natural and synthetic peptides of this portion of β-hCG react *in vitro* with hCG, but not

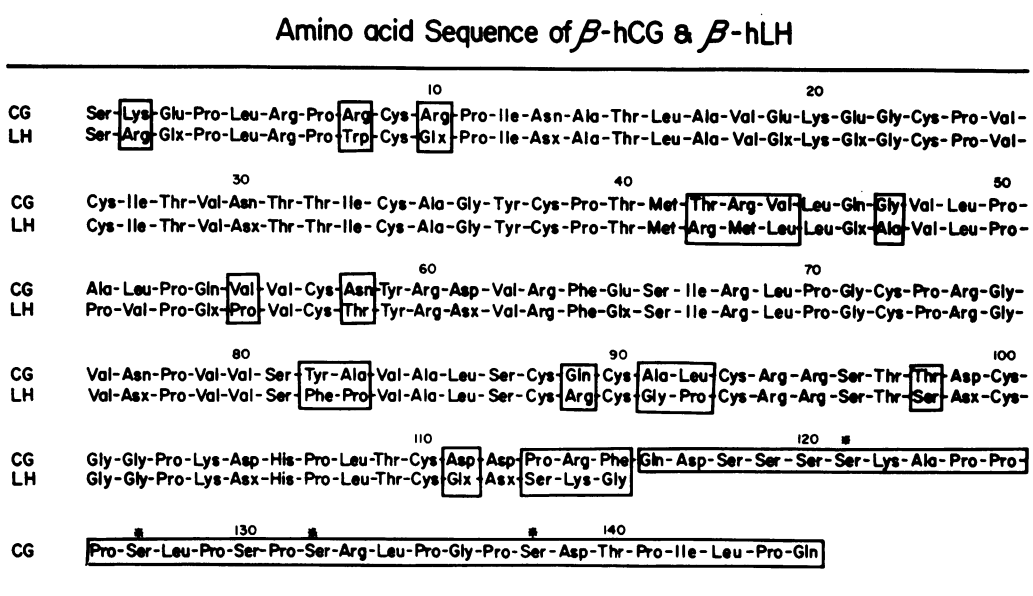

Fig. 2. Amino acid sequence of the beta subunits of human chorionic gonadotropin and human luteinizing hormone. Sequences in boxes indicate areas of difference in structure.

with hLH. Further, some of these antisera are capable of neutralizing hCG *in vivo*. Additional studies are in progress to define a carrier–peptide conjugate that not only renders these peptides more antigenic, but one that will also be safe for human application.

Recent studies have characterized the specificity of antibodies to C-terminal peptides of β-hCG. None of the studies have found a reaction to hLH or its subunits but a very low reactivity has been found with some substances in urinary extracts from nonpregnant women and acetone-dried human pituitary glands. To date, the nature of the material has not been determined and interpretation of these findings is difficult. If the antibodies used in the *in vitro* studies are absolutely specific for hCG, it must be concluded that hCG is produced in small quantities by normal tissues. While this is theoretically plausible, these same *in vitro* tests, using antipeptide sera, suggest that hCG is present in normal tissues of rats, dogs, sheep, and certain species of microorganism. Another explanation for these observations is that antipeptide antisera contain antibodies to determinants common to the hCG peptides and other body constituents. Since these detections of hCG-like material have been made from morbid tissue or urinary extracts, there is a possibility that reacting components are metabolic or denatured by-products of normal substances that are not present in the living organism. While some evidence for possible nonspecific reactions to immunization with hCG-β terminal peptides is available, careful *in vivo* assessment of the effects of animal immunization will reveal whether such therapy might constitute a hazard to human subjects. In order to obtain meaningful results from these studies the use of appropriate animal models and experimental designs are essential. Should these studies indicate damage to one or more nonreproductive tissues, suitable antigens may be found by preparing analogues to hCG peptides and thus avoiding cross-reactive determinants. While there are several factors yet to be evaluated before the utility of this approach can be

determined, the use of synthetic peptides of β-hCG appears to be promising for the development of an antifertility vaccine.

General studies

Besides the research directed toward identifying specific immunogens for vaccine development, other studies are in progress to support the application of methods or to facilitate the isolation of an appropriate material. One such effort is the establishment of the WHO Reference Bank for Reproductive Immunology. Sera from patients with unexplained infertility are collected in sufficient quantities to be distributed to scientists in the field who can evaluate them for the presence of antibodies to reproductive antigens. If some of these infertility patients were found to have generated spontaneously an immune response to one or more reproductive components, it might be possible to identify directly a suitable component for vaccine development. The sera may also be used to isolate antigens by employing them in affinity chromatography techniques. The Reference Bank is also attempting to accumulate samples of highly purified reproductive antigens in order to assist the detection of antibodies in the sera of infertile women. This approach may accelerate the overall effort to identify a suitable immunogen for vaccine development.

Research is also underway to determine whether an immune response can be generated by the female genital tract to block the events of fertilization or implantation. A local immune response by the vagina, cervix, uterus, or oviduct that produced a high concentration of antibodies near the site of their intended action, with little or no systemic immune response, would probably provide a much safer antifertility method. The immunocompetence of the female genital tract is virtually unknown and it is still uncertain whether such a method could be developed. The principal obstacle to research in this area is the lack of methods to deliver immunogens to the site of immunization. A practical procedure for local immunization must utilize a vaginal or cervical application that will be effective with infrequent treatments. The development of suitable delivery systems meeting these requirements would permit the evaluation of the feasibility of local immunization methods.

Another area of research that is important to antifertility vaccine development concerns immunological adjuvants. Since many reproductive antigens are weakly immunogenic, safe procedures to enhance immune responses would greatly accelerate progress in this work. Conventional adjuvants commonly used in animals, are not acceptable for human use because of the lesions produced at the site of injection and, in some cases, the use of toxic components. The effectiveness of these adjuvants depends upon a bacterial component and an oil component to retard clearance of the vaccine components. Recently, scientists have isolated certain bacterial components that effectively enhance immune responses without the undesirable reactions of conventional adjuvants. These materials can also be prepared synthetically and can be administered in aqueous vehicles. These developments will be of great value to the successful application of any antifertility vaccine.

SAFETY ASSESSMENT OF METHODS

In determining whether an effective antifertility vaccine is safe for human application, careful assessment must be carried out in a suitable animal model. The animal model used must be one whose fertility is inhibited by the vaccine, although the antibodies raised may

be stimulated by a human antigen. It is inappropriate to test the safety of a vaccine for potential human application in an animal species that has no endogenous antigens capable of cross-reacting with antifertility antibodies. Therefore, the species-specificity of a given antigen must be well known. Under most circumstances, the species used for safety evaluation should be a subhuman primate in order to obtain both efficacy and safety data most likely to resemble results in humans.

The possible hazards of antifertility vaccines can be divided into two categories: (1) problems related to immunization, and (2) problems caused by the antibodies produced. Since many of the suggested antigens for vaccine development are either weakly antigenic or are tolerant antigens in humans, chemical alteration and/or combination with adjuvants prior to injection may be required. The adjuvant selected would probably not contain natural bacterial components because of well known side-effects, such as disseminated granulomatosis and amyloidosis. Chemical compounds used for antigenic modification of antigens must be carefully tested for toxicity. Another aspect of the procedure needing close scrutiny is the production of IgE antibodies following immunization.

Theoretically, there are greater potential hazards from antibodies produced to reproductive antigens than from the immunization procedures. Particularly with hormone or enzyme antigens, cross-reactivity of antibodies to other hormones or enzymes must be carefully tested. Nonspecific neutralization of functional body substances could create severe problems, and certainly must be avoided. Secondly, any reaction of antibodies to maternal tissue components could lead to the establishment of autoimmunity, resulting in tissue damage. Critical assessment can be made by histopathological examination of all tissues likely to be affected from immunized animals of a species in which the vaccine was effective as an antifertility agent. Reactions of antibodies to tissue components, detected by immunofluorescence or other techniques, may counterindicate the use of a particular immunological method.

The formation of circulating antigen–antibody complexes as a result of antibody reactions with specific or non-specific antigens could result in immune complex disease. Certainly, any reaction of induced antibodies with maternal substances in slight antigen excess would create a hazardous situation. This phenomenon could also result from a reaction with a specific placenta, ovum, or sperm component upon its introduction into the blood or lymph if the substance were in slight antigen excess. Such immune complexes are usually small and during vascular circulation can become lodged in tissue spaces, such as along the glomerular basement membranes. These complexes can mediate tissue damage with or without the participation of complement. While histochemical studies of kidney tissue remain the definitive method for detection of immune complex disease, techniques for measuring soluble immune complexes in serum are available.

The potential problems and safety assessment procedures described above are certainly not exhaustive. Other hazards are theoretically possible, such as long-term effects on the genetic apparatus and immunological enhancement of malignancies. The summary presented here merely describes the first stage of the extensive studies that must be performed in the safety assessment of any antifertility vaccine.

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RÉSUMÉ

Approches immunologiques de la régulation de la fécondité

La mise au point d'un vaccin permettant une régulation de la fécondité exige la préparation d'antigènes bien définis chimiquement, indispensables à la reproduction, capables de provoquer une réponse immunitaire adéquate après injection à des sujets humains et dépourvus d'effets secondaires sur les systèmes autres que le système reproducteur.

L'approche immunologique trouve sa justification dans sa simplicité: application peu fréquente et sans recours à un personnel spécialisé, disponibilité pour un grand nombre de gens dans des régions dépourvues de cliniques ou d'autres ressources médicales, absence d'effets pharmacologiques secondaires. Pour obtenir un antigène qui convienne à la préparation d'un vaccin il faut *a*) que la substance à inhiber ne soit présente, chez le sujet vacciné, que par intermittence (par exemple, au cours de la grossesse ou des rapports); *b*) que la masse d'antigène à bloquer soit relativement faible; *c*) que la quantité d'antigène nécessaire à l'état purifié soit disponible à un coût raisonnable pour le rendre accessible à une population nombreuse.

La recherche moderne a porté son effort principal sur la mise au point de méthodes d'immunisation de la femme susceptibles de prévenir ou d'interrompre la grossesse. Le blocage des éléments constitutifs du placenta ou du sperme par une réponse immunitaire maternelle semble en effet un moyen sans danger d'intervenir sur la fonction reproductrice, ce qui n'est pas le cas dans l'inhibition de la fertilité masculine. Pour des raisons pratiques et de sécurité (sensibilisation, faible persistance des concentrations efficaces d'anticorps, nécessité d'injections fréquentes), la recherche s'est orientée vers la vaccination de préférence à l'immunisation passive. Mais la préparation d'un vaccin se heurte à une première grande difficulté qui est d'identifier et d'isoler un antigène étranger à l'organisme récepteur, par exemple spermatique ou placentaire, qui soit immunogène, c'est-à-dire qui engendre une réaction antifertilisante.

L'auteur fait un rapide bilan de l'état actuel des recherches sur les antigènes spermatiques, ovulaires et placentaires. Les antigènes spermatiques spécifiques solubles sont considérés comme les immunogènes les plus appropriés et parmi eux figurent les enzymes de surface du sperme. On a isolé et testé les enzymes suivantes: l'isoenzyme de la déshydrogénase lactate, la hyaluronidase des acrosomes et l'acrosine qui est une protéinase acrosomique. Les recherches effectuées avec ces enzymes et avec d'autres antigènes spermatiques ont été négatives. Les données rassemblées ont révélé la nature complexe de la membrane de surface du spermatozoïde dans laquelle on a identifié trente glycoprotéines. L'utilisation d'antigènes de l'ovule, moyen théorique idéal, se heurte jusqu'ici à divers obstacles sérieux: danger d'auto-immunisation et difficulté d'induire une antigénicité à un antigène de l'hôte. Les études préliminaires sur les antigènes du cumulus oophore et sur ceux des cellules de la couronne ont donné quelques résultats encourageants, mais celles effectuées sur la zone pellucide promettent davantage. L'isolement de quantités expérimentales d'antigènes spécifiques de cette zone permettrait d'aborder l'étude des questions essentielles: la spécificité des anticorps, leurs mécanismes de prévention de la fécondation et les conséquences de l'immunisation active et passive sur les antigènes mêmes de la zone pellucide. Les antigènes placentaires ont fait l'objet d'un important travail de recherche qui a porté sur l'utilisation d'anticorps envers les homogénats placentaires et de protéines placentaires spécifiques hautement purifiées, telles les

protéines SP-1 et PP-5. Les résultats du travail mené avec SP-1 (une glycoprotéine de p.m. proche de 90 000) laissent peu d'espoir quant à son emploi pour un vaccin, ceux qui ont été obtenus avec PP-5 justifient d'autres efforts. Trois protéines-hormones ont été isolées du tissu placentaire chorionique: la thyrotrophine, la gonadotrophine et la somatomammotrophine. Les pouvoirs antigéniques des deux dernières ont été étudiés. Leur manque de spécificité, en particulier leur ressemblance avec les hormones hypophysaires, sont un obstacle sérieux à leur emploi. L'expérimentation très poussée faite avec la fraction purifiée de la somatomammotrophine chorionique a donné des conclusions négatives. L'efficacité de la gonadotrophine chorionique en tant qu'immunogène a été bien établie, mais sa sécurité d'emploi sur l'animal même reste à explorer. L'examen des structures chimiques primaires des sous-unités bêta de cette gonadotrophine et de l'hormone lutéinisante hypophysaire suggère qu'il serait possible d'obtenir des anticorps antigonadotrophine spécifiques. Les études en cours visent à définir un composé conjugué porteur-peptide capable de rendre à la fois plus antigéniques les peptides constitutifs de la gonadotrophine et plus sûre leur application à la femme. A cette fin, le choix d'un modèle animal d'expérience adéquat est essentiel. Par ailleurs l'utilisation de peptides synthétiques ouvre une autre voie de recherche vers la mise au point d'un vaccin contre la fécondation.

L'approche immunologique réclame l'exploration d'avenues de recherche variées et l'organisation de services d'application des méthodes choisies ou d'isolement du matériel d'expérience nécessaire. Ainsi la Banque OMS de référence pour l'immunologie de la reproduction organise la collecte et l'étude de sérums de patients stériles et celle d'échantillons d'antigènes de la reproduction très purifiés. D'autres travaux portent sur la capacité du tractus génital féminin d'arrêter la fertilisation ou l'implantation, ou encore sur l'utilisation d'immuno-adjuvants sûrs et capables de stimuler une bonne réponse immunogène de la part des antigènes de la reproduction.

L'évaluation du degré de sécurité d'un vaccin anti-fécondation doit être faite sur un modèle animal approprié, c'est-à-dire, dont les anticorps peuvent être produits en réponse à un antigène humain et dont la fertilité est inhibée par le vaccin. Il importe donc de connaître le degré de spécificité d'espèce d'un antigène donné. L'espèce d'usage courant est un primate hominoïdé. L'utilisation de vaccins contre la fécondation comporte deux catégories de dangers: ceux qui concernent l'immunisation et ceux qui concernent les anticorps produits, ces derniers étant en principe plus graves que ceux-là. Ils peuvent donner lieu en effet: à des réactions croisées avec d'autres hormones ou enzymes, à une neutralisation non spécifique de constituants physiologiques, à des réactions avec les tissus maternels suivis d'un phénomène d'auto-immunité, à la formation d'immuncomplexes antigène-anticorps, etc. D'autres risques sont théoriquement possibles: les effets à long terme sur l'appareil génital et la stimulation immunitaire d'un état de malignité. La détection de ces risques met en jeu l'examen histopathologique, l'immunofluorescence ou d'autres techniques et apporte des renseignements sur le choix de la méthode d'immunisation à adopter.